Kidney International, Vol. 65 (2004), pp. 1511–1516

TECHNICAL NOTE

Micro-MRI methods to detect renal cysts in mice

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Micro-MRI methods to detect renal cysts in mice.

Background. Mouse models of disease, especially using transgenic and knockout technologies, are powerful tools to analyze the molecular basis of disease. We recently reported that a new dynamic micro-MRI method with dendrimer-based contrast agents can visualize renal structure and function in normal living mice and mice with acute renal failure. While MRI contrast enhancement is useful for detecting functional impairment of the kidneys, this technology has limitations in assessing morphologic changes, particularly cystic disease, because contrast-enhanced micro-MRI depicts cysts as low-intensity areas that cannot be distinguished from fibrotic foci.

Methods. In the current study, we evaluated if micro-MRI employing a new three-dimensional MR hydrography signal sequence [three-dimensional fast imaging employing steady-state acquisition (3D-FIESTA)] can visualize chronic cystic changes without any contrast agents.

Results. We were able to positively depict multiple renal cortical cysts of ~0.2 mm diameter in a mouse model of sickle cell disease and observe serial changes of renal cysts (>0.2 mm diameter) in cyclooxygenase-2 (COX-2) knockout mice during a $2^1/_2$-month period. Some cysts decreased in size over time.

Conclusions. Micro-MRI with 3D-FIESTA can depict cyst formation in the diseased kidneys of living mice without injection of contrast agents.

The major goal of renal imaging is to detect structural and functional abnormalities in vivo. The intrinsic contrast of the kidney parenchyma is low on MRI. We have recently shown that generation-4 (G4) dendrimer-based contrast agents are concentrated in renal tubules, enabling visualization of renal structural and functional injury in the mouse [1].

Chronic kidney disease is typically diagnosed by a combination of chronic interstitial inflammation/fibrosis and tubular atrophy, and is associated with cyst formation [2, 3]. While serum creatinine is a useful biomarker of chronic kidney disease in humans, serum creatinine is often not elevated in mouse models of renal disease [4]. Therefore, noninvasive detection of the changes in renal anatomy or function can play an important role in documenting chronic kidney disease in animal models. We employed a 1.5 Tesla clinical MRI system fitted with a mouse coil and used a new three-dimensional fast imaging employing steady-state acquisition (3D-FIESTA) to visualize renal cysts in two mouse models of chronic renal disease: sickle cell disease mice [3] and cyclooxygenase-2 (COX-2) knockout mice [2]. The 3D-FIESTA is a relatively new water-sensitive fast sequence (MR hydrography) that detects small amounts of fluid based on its long T2 relaxation [5–7]. This three-dimensional steady-state coherent imaging pulse sequence,3D-FIESTA, has recently become available on clinical MRI machines (Signa LX, GE, Milwaukee, WI, USA) and permits the acquisition of images with a 0.6 mm slice thickness while retaining high signal-to-noise ratios at very short scan times.

In the present study, we performed MRI-pathologic correlation studies on mice with chronic kidney diseases to evaluate the sensitivity of non-contrast enhanced 3D-FIESTA to detect cysts in mice with renal disease.

Key words: magnetic resonance imaging, kidney, chronic kidney disease, cyst, COX-2, sickle cell disease.

Received for publication May 30, 2003
and in revised form July 16, 2003, and September 4, 2003
Accepted for publication November 11, 2003

METHODS

Mouse models of experimental chronic kidney damage

The MRI methods were optimized in 2-month-old normal Balb/c mice (NCI, Frederick, MD, USA). For the models of chronic kidney disease with cysts, we used

8-month-old hemizygous sickle cell mice containing one normal mouse beta-globin allele ($N = 7$), and 5- and 10-month-old COX-2 knockout mice ($N = 4$, in each group). All studies were approved by the Animal Care Committee of National Institutes of Health.

Three-dimensional micro-MRI of the kidney

The 3D-FIESTA sequence was performed with 45° flip angle (TR/TE 27.7/13.8 msec; scan time 2 minutes 29 seconds; frequency encoding × phase encoding steps 320 × 224; 3 numbers of excitation; 16 slice encoding steps). The coronal images for MRI were reconstructed from 0.6 mm thick sections without gap. The field of view was 6 × 3 cm and the size of the matrix was 0.19 × 0.27 mm. In addition, we examined most of kidneys with two conventional MR hydrography sequences [2D-HASTE (two-dimensional half-Fourier acquisition single-shot turbo spin-echo); ssfse-IR TR/TE 12939/38.7 msec; TI 150 msec; scan time 13 seconds; frequency encoding × phase encoding steps 256 × 256; 1 number of excitation; field of view 8 × 8 cm] and 2D-STIR [(two-dimensional short tau inversion recovery); TR/TE 8000/102 msec; TI 150 msec; scan time 3 minutes 20 seconds; frequency encoding x phase encoding steps 512 × 256; 3 numbers of excitation; field of view 8 × 4 cm)]. 2D-HASTE method was not able to detect the kidney; whereas the 2D-STIR method could only depict subcortical cysts as vague white lines for the same kidney shown in Figure 2a (Fig. 2c and d). However, 3D-FIESTA could detect small renal cysts. We tested 3D-FIESTA and compared to contrast-enhanced three-dimensional fast-spoiled gradient echo (SPGR) (T1-weighted) with the G4-(1B4M-Gd)$_{64}$ dendrimer agent (0.03 mmol/kg) as previously described [1].

All scans were performed using a clinical grade 1.5 Tesla superconductive magnet unit (Signa LX) fitted with an in-house constructed 1-inch bird cage–type coil [1]. The mice were anesthetized with subcutaneous injection of 100 mg/kg of ketamine HCl (Fort Dodge, IA, USA) and placed in the center of the coils. Mice underwent T2-weighted noncontrast 3D-FIESTA imaging and then were intravenously injected with G4 dendrimer agent following the imaging by contrast-enhanced T1-weighted three-dimensional fast-SPGR.

RESULTS

Image quality of 3D-FIESTA in normal mice

We first compared the visualization of normal kidneys using 3D-FIESTA (Fig. 1a) with G4 dendrimer contrast-enhanced dynamic three-dimensional fast SPGR imaging in 18 consecutive mice ($N = 36$ kidneys). The G4 dendrimer agent is known to produce excellent imaging of the kidney [1]. All of the dynamic MRIs obtained with G4 agent had a uniform appearance, as seen previously, with

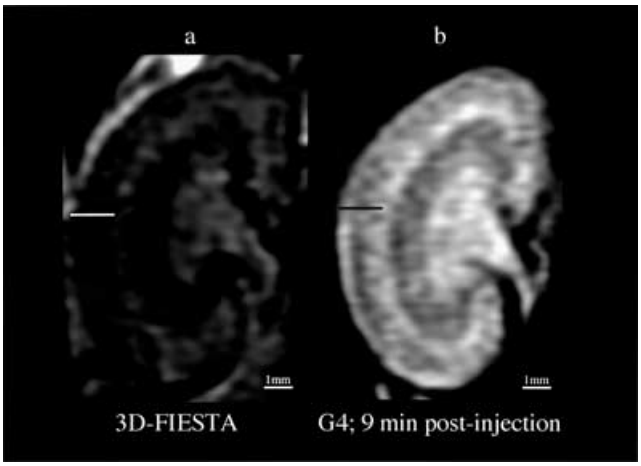

Fig. 1. The MR kidney images in a normal mouse with three-dimensional fast imaging employing steady-state acquisition (3D-FIESTA) (*a*) and T1-weighted G4 dynamic (*b*) 9 minutes postinjection are shown. The bars indicate the thickness of the cortex and outer stripe.

alternating bands of contrast in the cortex, outer stripe, and inner medulla [1]. In contrast, we found that the 3D-FIESTA method was susceptible to artifacts caused by air in the adjacent intestine; the kidney parenchyma was not visualized partially ($N = 5$) or totally ($N = 1$) in six of 36 normal kidneys. In order to simplify the experimental protocol, we did not repeat scans to obtain better images for this validation study (see **Discussion** section). In the remaining 30 kidneys, the parenchyma of the kidney was generally dark with a single thin faint bright band at the outer stripe of the outer medulla. The water (urine) in the pelvis was clearly depicted by 3D-FIESTA in all 30 kidneys as a bright area (see Fig. 1a).

3D-FIESTA can detect small renal cysts

We next evaluated the ability of these two methods to detect renal cysts in hemizygous sickle cell mice. The presence of these cysts was later confirmed by histologic analysis (Fig. 3).

The 3D-FIESTA images of sickle cell mice clearly showed bright cysts with diameters of ~0.2 mm, even when they were located near the renal capsule (Figs. 2 and 3). In contrast, whereas the dynamic MRI with G4 could detect internal cysts (dark circles), cysts near the surface of the kidney indistinctly appeared as a wavy surface contour, or were not detected (Figs. 2 and 3). Images taken 3 days apart showed good reproducibility (Fig. 4) (mean differences in size ± SD = 0.05 ± 0.02 mm; $N = 19$; $r^2 = 0.63$) of cyst diameter.

We also evaluated both MRI methods in COX-2 knockout mice. The 3D-FIESTA method could detect bright cysts in the inner stripe with a diameter of ~0.2 mm (Figs. 5 and 6). Although cysts were visible on G4 dynamic MRI, they were more easily identified on

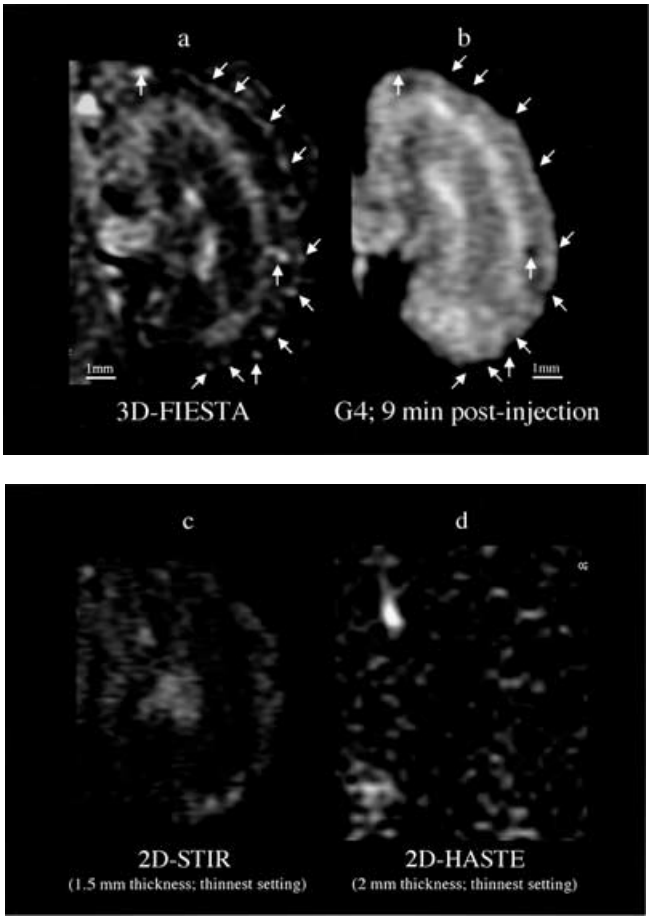

Fig. 2. The MR kidney images in a 8-month-old hemizygous sickle cell mouse with chronic kidney damage using three-dimensional fast imaging employing steady-state acquisition (3D-FIESTA) (*a*), T1-weighted G4 dynamic (*b*) 9 minutes postinjection, and two conventional MR-hydrography sequences [two-dimensional short tau inversion recovery (2D-STIR) (*c*) and two-dimensional half-Fourier acquisition single-shot turbo spin-echo (2D-HASTE) (*d*)] are shown. The arrows indicate multiple small cysts with diameter of ~0.2 to 0.3 mm.

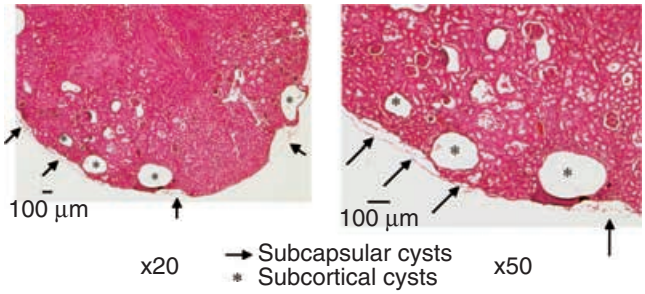

Fig. 3. Typical renal histology in a hemizygous sickle cell mouse. Arrows show subcapsular cysts; asterisks show subcortical cysts.

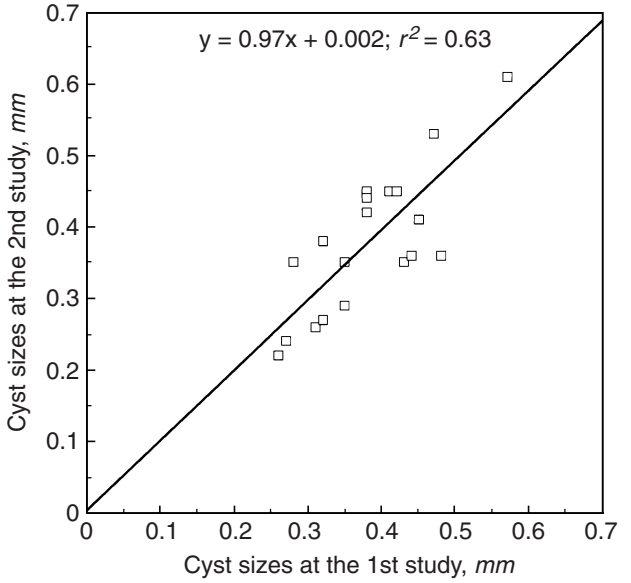

Fig. 4. Diameters of cysts in eight cyclooxygenase-2 (COX-2) knockout mice measured on two separate MRI scans with three-dimensional fast imaging employing steady-state acquisition (3D-FIESTA) method taken 3 days apart are plotted. Data show good reproducibility (mean differences in size ± SD = 0.05 ± 0.02 mm; $N = 19$; $r^2 = 0.63$).

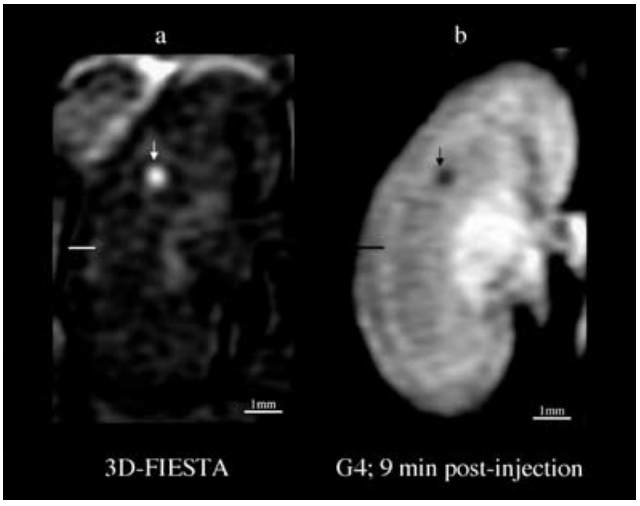

Fig. 5. The MR kidney images in a 10-month-old cyclooxygenase-2 (COX-2) knockout mouse with chronic kidney damage using three-dimensional fast imaging employing steady-state acquisition (3D-FIESTA) (*a*) and T1-weighted G4 dynamic (*b*) 9 minutes postinjection are shown. The arrow indicates a cyst with diameter of ~0.4 mm. The bars indicate the thickness of the cortex and outer stripe.

3D-FIESTA (Figs. 5 and 6). Serial MRI examinations at 5 and 7.5 months in one mouse showed that two cysts decreased their size from 5 to $7^1/_2$ months (Fig. 6). Serial MRI examinations in four mice showed that some cysts were stable, whereas others either increased or decreased in size. This decrease in size of four cysts was also found in the other COX-2 knockout mice (Fig. 7). Since the three-dimensional image acquisition covered the entire kidney parenchyma without gap, we would not miss a cyst of >0.3 mm as shown in Figure 6, although we could miss cysts of smaller than 0.1 mm diameter.

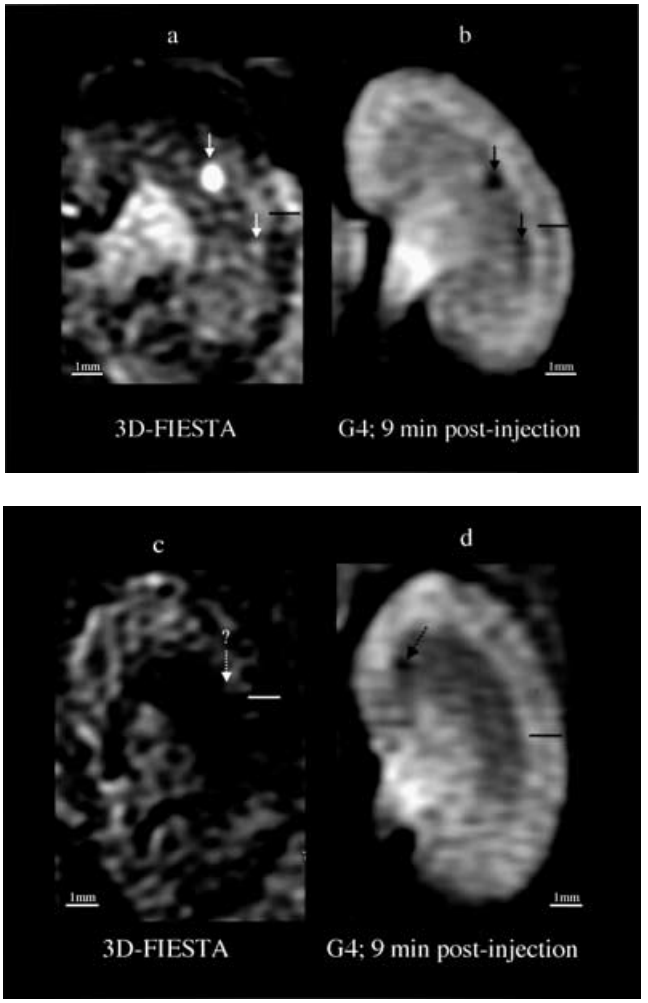

Fig. 6. The MR kidney images in a 5-month-old cyclooxygenase-2 (COX-2) knockout mouse with chronic kidney damage using three-dimensional fast imaging employing steady-state acquisition (3D-FIESTA) (*a*) and T1-weighted G4 dynamic (*b*) 9 minutes postinjection are shown. The arrow indicates a small cyst with a diameter of ~0.6 mm. The MR kidney images in the same COX-2 knockout mouse obtained $2^1/_2$ months later using 3D-FIESTA (*c*) and T1-weighted G4 dynamic (*d*) 9 minutes postinjection are shown. The cyst became almost nonvisualized on the images (a white broken arrow). The black broken arrow indicates nonenhancement area on the G4 image, which was not visualized by 3D-FIESTA. The bars indicate the thickness of the cortex and outer stripe.

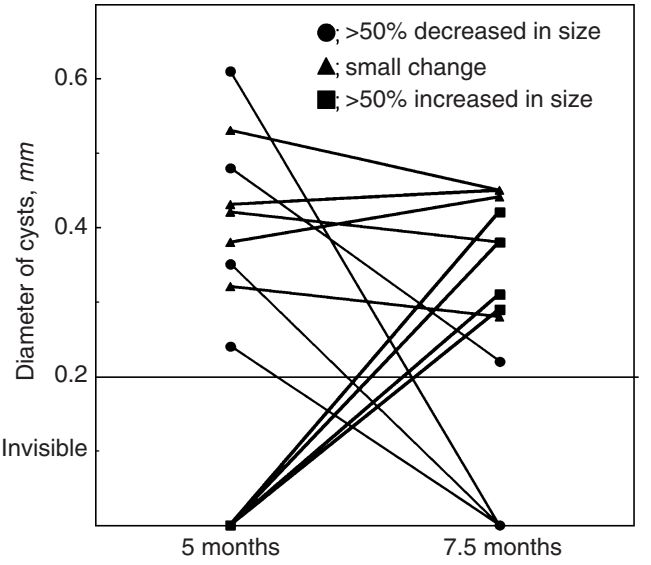

Fig. 7. The changes of cyst sizes in the kidneys in cyclooxygenase-2 (COX-2) knockout mice depicted by the serial three-dimensional fast imaging employing steady-state acquisition (3D-FIESTA) MR imaging during $2^1/_2$ months are plotted.

DISCUSSION

The 3D-FIESTA is a relatively new sequence for MR hydrography that has recently become available on clinical MRI units and that is highly sensitive for fluid-containing structures [8]. As demonstrated here, it can dramatically improve the conspicuity of renal cysts. Results using the FIESTA sequence have recently been reported in cardiac MRI and MR microscopy of cells [5, 6, 9]. To the best of our knowledge, this is the first reported use of 3D-FIESTA to analyze mouse models of kidney disease. As previously shown [10, 11], three-dimensional data acquisition has permitted generation of high-resolution micro-MR images in small animals. The inherent higher signal-to-noise ratio allows thinner 0.6 mm slices to be obtained as compared with the 1.5 mm slice typically of conventional two-dimensional data acquisition on a 1.5 Tesla unit. In addition, by using overlapping reconstruction, we can avoid a 0.2 mm gap between slices typical of two-dimensional data acquisition. Since the 3D-FIESTA method is very sensitive to the local water environment, 3D-FIESTA images were able to detect submillimeter renal cysts in mice with chronic kidney damage, even when the cysts were located on the surface of the cortex (Figs. 2 and 3). In addition, although all kidney MRI images were obtained twice within a 3-day interval, 3D-FIESTA rarely missed cysts with diameter more than 200 nm due to partial volume effects or slice gaps. Unfortunately, 3D-FIESTA images tended to be subject to susceptibility artifacts resultant from air or metal in the vicinity of the kidney. However, since colonic gas moves over time, one can potentially repeat the scan after a short time interval to obtain a better image (data not shown). Although we did not detect any hemorrhagic cysts by histology in this study, cyst hemorrhage might compromise the signal intensity of the water contained in the cyst because of susceptibility derived from iron ions incorporated in the hemoglobin.

3D-FIESTA has several advantages over contrast-enhanced imaging. First, 3D-FIESTA depicts cysts as bright areas against a dark background, which are more easily detected than dark areas against a bright background as visualized in contrast-enhanced imaging. Second, a bright circle on a FIESTA scan is more specific for

a cyst. For example, fibrosis or calcified lesions may also show up as low intensity areas on contrast-enhanced T1-weighted contrast images. Therefore, G4-enhanced MRI showed many spotty low intensity areas, but only some of them were cysts by 3D-FIESTA. In contrast, since 3D-FIESTA only visualizes water-rich areas with high signal intensity, high signal areas indicate the presence of cysts or urine (inner medulla). In addition, 3D-FIESTA method depicted cysts better than conventional MR hydrography methods such as 2D-HASTE and 2D-STIR. Third, and perhaps most important, 3D-FIESTA imaging does not require injection of an intravenous contrast agent.

Sun et al [12] have recently reported an MRI study of the polycystic kidney model mouse with an 8.5 Tesla animal MRI system. In their study, the affected kidneys were studied using a two-dimensional proton-weighted MRI. The proton-weighted MRI did not specifically detect individual cysts, although it did show segmentation of cysts and enlargement of the kidney. Because 3D-FIESTA has a high signal-to-noise ratio, we only needed a clinical 1.5 Tesla MRI scanner to obtain 0.6 mm slices, whereas Sun et al [12] used a research-grade 8.5 Tesla MRI machine to obtain 1.0 mm slides. Furthermore, since high signal on 3D-FIESTA is specific for fluid-containing structures, 3D-FIESTA might be useful for evaluating the serial volume fraction changes, and with image reconstruction, the total volume of renal cysts in polycystic kidney mice.

However, since the boxel size in this study was not isometric ($0.19 \times 0.27 \times 0.6$ mm), the volume of an individual cyst might be overestimated because the cyst is smaller than the z-axis resolution (0.6 mm). Accurate volumetry of the individual cyst might be accomplished by a smaller isometric boxel acquisition using the FIESTA method on the higher magnetic field machine with longer acquisition time. Therefore, in the current study, we measured the diameter of the cyst in the x-y plane. The reproducibility of images taken twice within 3 days was very good.

Another feature of the 3D-FIESTA sequence is its dependence on flip angle. Large flip angles produced more water signal but less tissue contrast. At flip angles of $>45^\circ$, the kidneys became too dark to accurately localize cysts. Intermediate flip angles (e.g., 45°) enabled visualization of both the kidney and the cysts. In the case of polycystic kidney where most of the renal parenchyma is replaced with cysts, a greater flip angle might help to measure the total volume of water in the cysts.

G4 agent–enhanced MRI is good at visualizing the structure and function of the cortex and outer stripe [1]. We found that the cortex plus outer stripe were thinner in COX-2 knockout mice than in the littermates. This finding has been reported as one of characteristic effects of the COX-2 gene deletion to the structure of the kidney [2].

Rodent models have been used extensively to study the pathophysiology of renal cystic diseases, especially polycystic kidney disease. Rodent models have also been used for preclinical drug discovery studies, with evidence for inhibition of renal dysfunction and cyst growth using inhibitors of angiotensin-converting enzyme (ACE), tumor necrosis factor (TNF), and epidermal growth factor (EGF), and potassium citrate, and soyasaponin [13–17]. Most of these studies relied on plasma markers of renal function and cyst size at the time of sacrifice. Patients whose kidneys become enlarged are at higher risk of developing renal failure, suggesting that monitoring cyst size might be used to monitor the progression of polycystic kidney disease [18]. Since MRI can noninvasively measure cyst size in live mice, it will be much easier to determine the natural history of each cyst. Furthermore, it may be possible to screen for drug effects much more rapidly, since cyst size can be used as a surrogate marker of drug effect. Finally, MRI will be useful for detecting subtle structural renal defects in enu-mutagenesis projects that are currently underway.

CONCLUSION

The micro-MRI method with noncontrast 3D-FIESTA enabled visualization of renal cysts in living mice to study changes in individual cysts over time. Therefore, this micro-MRI method should greatly aid in the non-invasive investigation of chronic kidney damage in mouse models, which cause cystic disease of the kidneys.

ACKNOWLEDGMENT

This study was supported in part by the National Cancer Institute (contract NO1-CO-12400).

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